(0.35 mg/kg) had a similar but much more marked effect (table).

Discussion. Douglas et al.[10] observed a reduced effect of i.v. and aerosolized histamine on the dynamic compliance of urethane-anesthetized as compared to conscious guinea-pigs. Our results agree well with these findings, and demonstrate that urethane has a direct depressive effect on histamine-induced contractions of guinea-pig smooth muscle at some step(s) beyond H_1 receptor activation.

The observation that, even when administered in subanesthetic doses, aerosolized urethane produces a delay in histamine-induced bronchospasm, indicates that, under appropriate experimental conditions, the direct effect of urethane on histamine-induced contractions of bronchial smooth muscle is a depressive one. In fact the 'topical' application of aerosolized urethane rules out the intervention of a centrally-mediated[11] reduction of sympathetic bronchodilator tone[1].

Urethane-induced depression of tracheal smooth muscle resting tone in vitro could likewise indicate that the increase in pulmonary airway resistance in urethane anesthetized as compared to conscious guinea-pigs depends upon an indirect effect of this anesthetic[1].

Urethane antagonism toward cardiovascular responsiveness to noradrenaline[3-6] and chronotropic response to isoprenaline[12] could be explained by an inhibition of agonist induced transmembrane Ca^{++} influx[13]. This mechanism could likewise explain our observation concerning the depressive effect of urethane on histamine-induced contraction of guinea-pig tracheal smooth muscle. In fact: a) unlike chlorpheniramine, a competitive H_1 receptor antagonist[8], urethane proved to be a non competitive antagonist, b) unlike chlorpheniramine, urethane antagonized $CaCl_2$-induced contractions in a high K^+ Ca^{++} free medium (a similar effect has been observed in rat aorta and portal vein[13]); c) urethane but not chlorpheniramine antagonized high K^+-induced contractions, which appear to be produced by a voltage-dependent activation of Ca^{++} channels in the cell membrane[14] leading to an inward movement of Ca^{++} responsible for smooth muscle contraction[15].

In addition, histamine-induced contractions of guinea-pig tracheal smooth muscle are known to be closely dependent upon the influx of Ca^{++} from the extracellular space, since they are markedly depressed in Ca^{++} free medium[16].

In conclusion, the direct effect of urethane on histamine-induced contractions of guinea-pig tracheal smooth muscle both in vitro and in vivo is of a depressive type, and it could be tentatively attributed to an interference with some basic cellular mechanism of Ca^{++} transmembrane movement.

1 Advenier, C., Boissier, J.R., Ho, S., Hallard, B., and Ruff, F., Br. J. Pharmac. *64* (1978) 519.
2 Volicer, L., and Loew, G.G., Pharmacology *6* (1971) 193.
3 Bunag, R., and Mullenix, P., Br. J. Pharmac. *46* (1972) 511.
4 Brezenoff, H.E., Br. J. Pharmac. *49* (1973) 565.
5 Miller, F.N., and Wiegmann, D.L., Eur. J. Pharmac. *44* (1977) 331.
6 Armstrong, J.M., Br. J. Pharmac. *74* (1981) 826P.
7 Van Rossum, J.M., Archs int. Pharmacodyn. Ther. *143* (1963) 299.
8 Castillo, J.C., and De Beer, E.J., J. Pharmac. exp. Ther. *90* (1947) 104.
9 Litchfield, J.T., and Wilcoxon, F., J. Pharmac. exp. Ther. *96* (1949) 99.
10 Douglas, J.S., Dennis, M.W., Ridgway, P., and Bouhuys, A., J. Pharmac. exp. Ther. *180* (1972) 98.
11 Bunag, R.D., and Eferakeya, J.E., Pharmacology *10* (1973) 143.
12 Maggi, C.A., and Meli, A., Experientia *38* (1982) 517.
13 Altura, B.M., and Weinberg, J., Br. J. Pharmac. *67* (1979) 255.
14 Bolton, T.B., Physiol. Rev. *59* (1979) 606.
15 Hurwitz, L., Mc Guffee, L.J., Little, S.A., and Blumberg, H., J. Pharmac. exp. Ther. *214* (1980) 574.
16 Creese, B.R., and Denborough, M.A., Clin. exp. Pharmac. Physiol. *8* (1981) 175.

Is vitellogenin a cuticular component of the female locust?

M. Papillon and P. Cassier

Université Pierre et Marie Curie, ERA 070620, 105, boulevard Raspail, F-75006 Paris (France), 18 December 1981

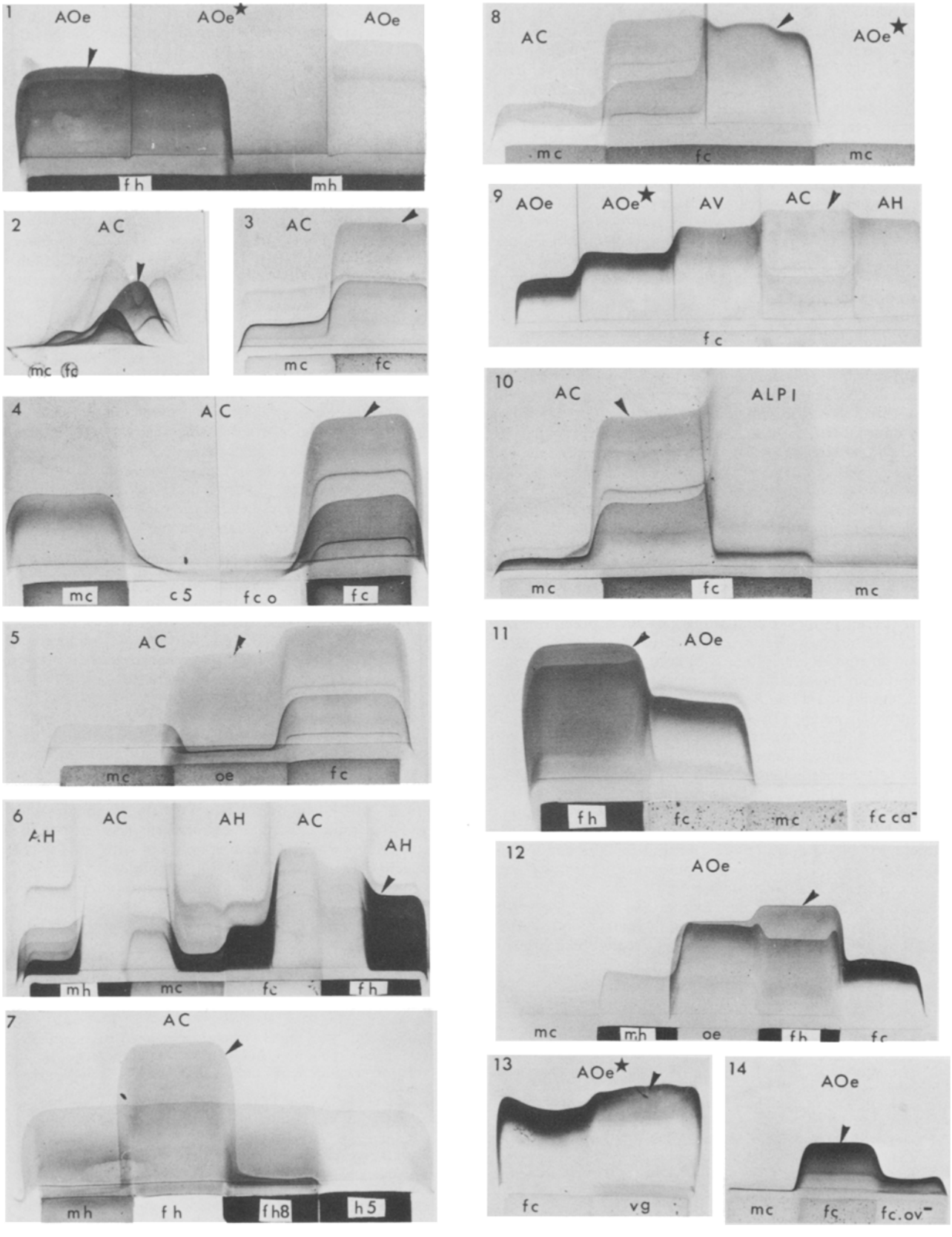
Summary. One major antigen, present in female cuticle, female blood and eggs, is revealed by the antiserum against soluble cuticular proteins of adults locusts, and by all the antisera against vitellin or vitellogenin. It is not revealed by the specific antiserum against diglyceride-binding lipoprotein. The presence of this major antigen in the cuticle depends on the presence of vitellogenin in the blood.

In insects, proteins are important cuticular components[1,2]. While the question of their site of synthesis remains unanswered, there is some evidence that a relationship exists between the hemolymph and cuticle proteins. For instance, in *Locusta migratoria*[3], *Periplaneta americana*[4], *Manduca sexta*[5], and *Calliphora vicina*[6], radiotracer studies have shown that some blood proteins can be transported into the cuticle; moreover, it has been demonstrated by immunodiffusion techniques that some cuticular proteins are immunologically similar to blood proteins[5,7,8]. The existence of sex-linked proteins in the cuticle of adults was reported for the first time in *Locusta migratoria*[9] by means of electrophoretic and immunodiffusion analysis.

The aim of our study was to elucidate the origin of one major female specific cuticular protein.

Material and methods. Rabbit antisera against soluble cuticular proteins of adult male and female locusts (AC), blood of adult females (AH) and eggs (AOe) were prepared according to the immunization schedule of Harboe and Ingel[10]. The supernatant (AOe*) of the AOe serum precipitated with male blood was equivalent to antivitellin (fig. 1). Specific antivitellin (AV) and specific anti-diglyceride-binding lipoprotein (A-LPI) sera were kindly given to us by Proff. Appelbaum and Emmerich, respectively.

Abdominal cuticles were carefully cleaned from adhering tissues, blood and cells, with small pieces of cotton mois-



Figures 1–14. Immunoelectrophoretic studies of cuticular proteins. Antibodies: polyvalent antisera against cuticular proteins of adult locusts (AC), hemolymph of adult females (AH), egg extracts (AOe); specific antisera against vitellin (AV), diglyceride binding-lipoprotein (A LPI); antiserum against egg extracts previously precipitated by male hemolymph (AOe*). Antigens: cuticular extracts of 5th instar larvae (c5), 0–1-day-old females (fco), adult females (fc), allatectomized females (fc ca$^-$), ovariectomized females (fc ov$^-$), adult males (mc); hemolymph supernatants of 5th instar larvae (h5), 8-day-old females (fh8), adult females (fh), adult males (mh); egg extracts (oe); purified vitellogenin (vg). Arrowhead: 'A' precipitation line.

tened with 0.4 M NaCl, quickly washed in 0.4 M NaCl solution, dried and ground to dust in liquid nitrogen. The cuticle powder was then mixed with phosphate buffer (0.01 M; pH 7.2) by stirring at 4 °C for 48 h. After centrifugation (9000 × g for 30 min at 4 °C), the supernatant was lyophilized and stored at −20 °C. It was dissolved in the electrophoresis buffer (Tris 0.047 M; barbital 0.013 M; Na-barbital 0.047 M; pH 8.8). Other antigens, such as eggs and blood were directly mixed with the same buffer and centrifuged. Methods used by us (crossed, tandem-crossed and line-immunoelectrophoresis) have been described by Weeke[11] and Krøll[12,13]. The vitellogenin was purified from ovariectomized females blood by the procedure of Chinzei et al.[14]. Protein extracts prepared from cuticles of 6–8-week-old male and female locusts are referred to as 'adult protein cuticular extracts'.

Results and discussion. When crossed immunoelectrophoresis of adult male and female cuticular protein extracts was performed in a gel containing serum against cuticle (AC), 3 main double peaks were visualized, and 1 important peak (A) formed from the female sample lacks a corresponding male component; however, that A precipitate is not symmetrical, since one of its feet deviates towards the male well (fig. 2).

When the same samples and anti-serum were used in line-immunoelectrophoresis, the most important precipitate issuing from the female cuticle was in continuity with a male precipitate, which is close to the starting line (fig. 3) or is deviated towards the male extract (figs 8, 10). When protein extracts of cuticles obtained from sexually mature males and females, young females (0–1 day old) and 5th instar larvae were run in AC containing gel, the A precipitin line appears to be characteristic of adult female extracts (fig. 4). Moreover, the A precipitate is in continuity with the main precipitation line arising from egg extract (fig. 5) and from adult female blood (fig. 6).

When blood samples from adult male, adult female, young female (5–6 days old) and 5th instar larvae were run into the AC gel, 2 main immunoprecipitates were observed, 1 of which (A) developed from adult and young females blood only (fig. 7). The A antigen disappeared when the AC serum has been previously precipitated with female blood.

From these observations, it can be concluded that the cuticle, the blood and the eggs of adult females contain the A antigen, as revealed by immunoprecipitation by antiserum against cuticular proteins.

The migration of cuticular extracts in anti-eggs serum which was previously precipited with male blood (AOe*) has shown that the A antigen is also revealed by antiserum against vitellin and is present in female cuticle only (fig. 8). In the same serum (AOe*), female cuticular extract and purified vitellogenin gave rise to only one precipitate, the A antigen (fig. 13). Electrophoresis of protein extract from adult female cuticle was performed in adjoining gels containing the following antisera AOe, AOe*, AV, AH and AC. The continuity of one precipitin line was obvious in all the antisera used. This line has the same position in AC as that A line already observed (fig. 9).

When blood and cuticle of male and female adult locusts were run into adjoining AC and AH gels, the A precipitate arose from both female blood and cuticle, and it was revealed by the 2 antisera; however there was no corresponding male precipitate (fig. 6). The A-LPI serum did not react with the antigen which was revealed by the AC serum (fig. 10).

It appears that the A antigen, present in female cuticle, female blood and eggs, is revealed by all the antisera against antigens which contain vitellin or vitellogenin but is not revealed by the A-LPI.

The migration of cuticular extracts in AOe containing gel has shown that the A antigen was present in the cuticle of ovariectomized females (fig. 14) which have high titers of JH III[15] and vitellogenin in their blood but no detectable amounts of ecdysteroids (Porcheron, personal communication). Moreover, the A antigen disappeared from the cuticle of allatectomized females whose blood contained neither JH III nor vitellogenin (fig. 11). Our results indicate that the presence of the A antigen in the cuticle depends on the presence of vitellogenin in the blood.

When male and female cuticular proteins, male and female blood as well as egg extract were run in AOe gel, 2 main precipitates were visualized: one of them corresponded to the A antigen, while the other one developed from eggs and blood only (fig. 12). It can be concluded that a protein component can occur in large amounts in the blood without being incorporated into the cuticle.

These results show that a female cuticular protein is immunologically identical with vitellogenin and vitellin, and becomes a cuticular component when all the conditions required for vitellogenin synthesis are met. The fact that in AC serum, the A precipitate of female cuticle is either in continuity with a male cuticular antigen, or deviated towards the male cuticular extract, suggests a possible linkage of this vitellogenin-like protein with a cuticular component present in both males and females.

Studies which are in progress in our laboratory (electrophoresis, protein separation procedures, incorporation of labelled precursors) may provide more data concerning the identity of the female cuticular protein.

1 Cassier, P., Porcheron, P., Papillon, M., and Lensky, Y., Annls Sci. nat., Zool. *2* (1980) 51.
2 Phillipps, D.R., and Loughton, B.G., J. Insect Physiol. *27* (1981) 475.
3 Tobe, S.S., and Loughton, B.G., J. Insect Physiol. *15* (1969) 1331.
4 Geiger, J.G., Kroalak, J.M., and Mills, R.R., J. Insect Physiol. *23* (1977) 227.
5 Koeppe, J.K., and Gilbert, L.I., J. Insect Physiol. *19* (1973) 615.
6 Scheller, K., Zimmermann, H.P., and Sekeris, C.E., Z. Naturforsch. *35C* (1980) 387.
7 Fox, F.R., Seed, J.R., and Mills, R.R., J. Insect Physiol. *18* (1972) 2065.
8 Phillipps, D.R., and Loughton, B.G., Comp. Biochem. Physiol. *55B* (1976) 129.
9 Lensky, Y., and Cassier, P., C. r. Acad. Sci. Paris, D*287* (1978) 515.
10 Harboe, N., and Ingild, A., in: A manual of quantitative immunoelectrophoresis. Methods and applications, p. 161. Eds N.H. Axelsen, J. Krøll and B. Weeke. Universitetsforlaget, Oslo 1975.
11 Weeke, B., in: A manual of quantitative immunoelectrophoresis. Methods and applications, p.47. Eds N.H. Axelsen, J. Krøll and B. Weeke. Universitetsforlaget, Oslo 1975.
12 Krøll, J., in: A manual of quantitative immunoelectrophoresis. Methods and applications, p.57. Eds N.H. Axelsen, J. Krøll and B. Weeke. Universitetsforlaget, Oslo 1975.
13 Krøll, J., in: A manual of quantitative immunoelectrophoresis. Methods and applications, p.61. Eds N.H. Axelsen, J. Krøll and B. Weeke. Universitetsforlaget, Oslo 1975.
14 Chinzei, Y., Chino, H., and Wyatt, G.R., Insect Biochem. *11* (1981) 1.
15 Johnson, R.A., and Hill, L., J. Insect Physiol. *21* (1975) 1517.